The *Bacillus subtilis* *dnaI* gene is part of the *dnaB* operon

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The *dnaI* gene of *Bacillus subtilis*, previously identified through the isolation of the *dnaI2* mutant, was found to be the second gene of the *dnaB* operon. The nucleotide substitution in the *dnaI2* mutant gene was determined.

Keywords: *Bacillus subtilis*, *dnaI* gene, replication

Many genes of *Bacillus subtilis* involved in DNA replication have been identified through the isolation of thermosensitive mutants. One of the mutations, *dnaI2*, affects an unknown step of chromosome replication at the restrictive temperature (Karamata & Gross, 1970). At the permissive temperature, the mutation affects lagging strand DNA synthesis of plasmids derived from pAM β 1, as judged by the accumulation of single-strand DNA, corresponding to the lagging strand template of the plasmid. In addition, a primosome assembly site present on pAM β 1, detected by its ability to prevent accumulation of single-strand DNA of a rolling circle plasmid, is no longer active in *dnaI2* cells (C. Bruand, S. D. Ehrlich & L. Jannire, unpublished data). Taken together, these data suggest that the *dnaI* gene product is a component of a *B. subtilis* primosome.

The *dnaI* gene was previously mapped around 250° on the *B. subtilis* chromosome (Karamata & Gross, 1970). We confirmed this position by showing that the gene is carried by the DNA insert of YAC 15-722 (Azevedo *et al.*, 1993), since this YAC transformed *B. subtilis* *dnaI2* cells (strain L1439; *dnaI2 metC ilvA*; Mael & Karamata, 1984) to the Dna⁺ phenotype (i.e. growth at 50 °C). Another gene involved in DNA replication, *dnaB*, was localized in the insert of YAC 15-722 by DNA hybridization (Azevedo *et al.*, 1993). This gene was previously cloned with its flanking regions into plasmid vectors, and its nucleotide sequence was determined (Ogasawara *et al.*, 1986; Perego *et al.*, 1987; Hoshino *et al.*, 1987). We wondered whether the *dnaI* gene was co-cloned with *dnaB* in these plasmid constructs and tested their ability to transform *dnaI2* cells for the Dna⁺ phenotype. We found that plasmids pMP66-29 and pMP33-25 do carry the *dnaI* gene (Fig. 1). Using various deletion derivatives of these plasmids (p1–p4 in Fig. 1), we found that the *dnaI* gene is encoded by the ORF located immediately downstream of *dnaB* (previously called ORF311 or ORFY or *dnaY*; Ogasawara *et al.*, 1986; Hoshino *et al.*, 1987).

The results obtained with constructs p3 and p4 (Fig. 1) suggested that the *dnaI2* mutation was located in a ~320 bp segment. This was confirmed by showing that a slightly larger segment (372 bp), generated by PCR from wild-type cells (strain L1430; *lys-21 metC ilvA*), transformed *dnaI2* cells to the Dna⁺ phenotype when present on a pUC19 plasmid derivative (plasmid p5; Fig. 1). In contrast, the same segment derived from *dnaI2* cells did not transform. The nucleotide sequence of the wild-type and mutant segments was determined. The only difference between the two was a G to A substitution at

Fig. 1. Schematic representation of a 5.3 kb region of the *B. subtilis* chromosome containing the *dnaB* and *dnaI* genes. Arrows indicate ORFs and direction of transcription deduced from the nucleotide sequence (Ogasawara *et al.*, 1986; Hoshino *et al.*, 1987). Open bars designate the DNA segments able (+) or not able (–) to transform *dnaI2* cells to thermoresistance (growth at 50 °C). These segments were carried by plasmids unable to replicate in *B. subtilis*, that were constructed as follows: pMP66-29 and pMP33-25 were described previously (Perego *et al.*, 1987), p1 and p2 were derived from pMP66-29 by *EagI* and *XcmI*–*SphI* deletions, respectively, and p3 was derived from pMP33-25 by an *EcoRI* deletion. p4 was derived from p1 by a *XcmI*–*NheI* deletion. For plasmid p5, the DNA segment was generated by PCR from wild-type cells, using 5' GCCTGAATTTGTGAGAGAGC and 5' GTTCCTTCCCCTTGAACC as primers. This segment was then cloned into the *HindIII*-cut plasmid pHV2901 (a pUC19 derivative; Chdin *et al.*, 1994).

position 2756 (coordinates as in Ogasawara *et al.*, 1986), which leads to a glycine to glutamate change. The change occurs seven residues from the end of the protein, outside the putative nucleotide binding motif (Ogasawara *et al.*, 1986).

dnaI is co-transcribed with the neighbouring genes *dnaB* and ORF213 (Sueoka *et al.*, 1988; Laffan & Sueoka, 1991). Laffan & Sueoka (1991) reported that antibodies raised against synthetic peptides derived from the sequence of all three genes inhibited the initiation of chromosome replication in an *in vitro* system derived from a membrane complex. Our finding that ORFY corresponds to *dnaI* lends further evidence to its involvement in DNA replication.

The products of genes *dnaB* and *dnaI* are assumed to be components of a *B. subtilis* primosome (C. Bruand, S. D. Ehrlich & L. Jannire, unpublished data). The third gene of the operon might therefore also be involved in primosome activity. It is interesting to note that these proteins, as well as another putative primosome component, *dnaD*, do not have any known homologues in *Escherichia coli* (Hoshino *et al.*, 1986; Ogasawara *et al.*, 1987; Bruand *et al.*, 1995). It is possible that homologous genes do exist in *E. coli* but are still unknown, in spite of the extensive studies on DNA replication in this organism. An alternative is that proteins with analogous functions exist, but without any similarity at the amino acid level, as observed for other replication proteins (Stillman, 1994). Finally, these genes could be specific to DNA replication in *B. subtilis*.

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